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Nutrition 21 (2005) 686–690

NUTRITION

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Applied nutritional investigation

Prediagnostic plasma vitamin C levels and the subsequent risk of prostate cancer

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Manuscript received January 8, 2004; accepted November 2, 2004.

Abstract

Objective: Antioxidants, such as vitamin C, are hypothesized to prevent prostate carcinogenesis by protecting the DNA from oxidative damage. We assessed whether higher prediagnostic plasma concentrations of vitamin C were associated with a lower risk of prostate cancer in a well-nourished cohort of men.

Methods: Plasma concentrations of ascorbic acid (vitamin C) were previously determined in blood specimens collected between 1984 and 1990 in men participating in the Baltimore Longitudinal Study of Aging. Total plasma ascorbic acid (L-ascorbic acid plus dehydro-L-ascorbic acid) levels were measured by using a modification of the 2,4-dinitrophenylhydrazine method. Among the 498 male participants with measured plasma vitamin C levels, 62 men were subsequently diagnosed with prostate cancer during their lifetime. Cox proportional hazards regression models were used to estimate relative risks and 95% confidence intervals for prostate cancer.

Results: The median plasma concentration of vitamin C for the cohort was 1.17 mg/dL, which is in the normal to high range for older men. The age-adjusted relative risk of prostate cancer for the highest quartile (median = 1.47 mg/dL, range = 1.36–2.58) compared with the lowest quartile (median = 0.83 mg/dL, range = 0.15–0.98) of plasma vitamin C concentration was 1.31 (95% confidence interval 0.63 to 2.70, P for trend = 0.29). Adjustment for cigarette smoking status, body mass index, or plasma cholesterol concentration did not attenuate the results.

Conclusions: This small but prospective study suggests that higher plasma vitamin C concentrations within the normal physiologic range are not associated with a lower risk of prostate cancer in well-nourished men.

Keywords: Antioxidants; Vitamin C; Plasma; Prostate cancer; Cohort study

Introduction

Oxidative stress is thought to play an important role in prostate carcinogenesis. Oxidative stress is caused by an abundance of reactive oxygen species that are generated from endogenous processes and exposure to exogenous factors. Androgens may contribute to development of prostate cancer by generating reactive oxygen species [1], which can damage DNA and lead to DNA mutations. Antioxidants are hypothesized to protect against carcinogenesis by scavenging free radicals and protecting the DNA from oxidative damage.

Vitamin C (ascorbic acid) is a water-soluble antioxidant that has the potential to prevent prostate cancer based on evidence from laboratory studies. Experimental studies

This work was supported by Prostate SPORE NCI-CA58236, a grant from the Laffey-McHugh Foundation, and by the NIA Intramural Research Program. Hoffmann-LaRoche, Inc. provided funding for plasma analyses. Dr. Berndt was supported by NCI NRSA grant 5-T32-CA09312. Dr. Platz is supported by the Bernstein Young Investigator's Award.

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0899-9007/05/$ – see front matter
doi:10.1016/j.nut.2004.11.012

have demonstrated that vitamin C inhibits the growth of androgen-independent and androgen-dependent prostate cancer cells in vitro [2] and decreases the production of reactive oxygen species in androgen-treated prostate cancer cells [1]. In addition, plasma vitamin C has been shown to be inversely correlated with biomarkers of oxidative stress even after adjusting for other antioxidants [3], suggesting that vitamin C may decrease oxidative stress. Prospective studies examining blood levels [4,5] or dietary intake [6–9] of vitamin C and prostate cancer risk have not observed a significant protective association. However, two retrospective case-control studies found that higher dietary intake of vitamin C was associated with a decreased risk of prostate cancer [10,11].

To elucidate further the relation between vitamin C and prostate cancer, we examined prediagnostic plasma levels of vitamin C in men participating in the Baltimore Longitudinal Study of Aging (BLSA). We hypothesized that high plasma vitamin C concentrations would be associated with a lower risk of subsequent prostate cancer.

Materials and methods

Subjects

The BLSA is an ongoing cohort of men and women that has continuously enrolled participants since 1958 and presently includes 1806 men. The participants are community-dwelling, predominately middle class, and white (87%). Details of this study population have been published elsewhere [12]. In brief, participants are given a comprehensive medical, physical, and neuropsychological examination approximately every 2 y at the National Institute on Aging, Gerontology Research Center in Baltimore, Maryland. The institutional review boards of the Johns Hopkins Bayview Medical Research Center and the Gerontology Center approved the BLSA prostate protocol, and all subjects gave written informed consent for their participation.

Plasma analysis

As part of the nutritional assessment component of the BLSA study protocol, fasting plasma vitamin C (ascorbic acid) concentrations were measured previously in blood samples drawn from participants between 1984 and 1990 [13]. Blood was collected in heparinized evacuated tubes, mixed, and centrifuged at 4°C. An equal volume of 10% metaphosphoric acid was used to deproteinize the plasma samples for analysis. Samples were then frozen at −70°C and shipped overnight on dry ice to a reference laboratory (Edward Norkus, Our Lady of Mercy Medical Center, New York, NY, USA). Total ascorbic acid (L-ascorbic acid plus dehydro-L-ascorbic acid) was determined by using a modification [14] of the 2,4-dinitrophenylhydrazine method [15]. Plasma vitamin C concentrations were available for 515 men ages 21 to 96 y.

Identification of prostate cancer cases

The diagnosis of prostate cancer was confirmed by histology or established by clinical history and medical chart review. Of the 515 men with plasma vitamin C concentrations available, 92 had a diagnosis of prostate cancer. Thirteen cases were discovered only on autopsy. To limit the opportunity for detection bias, the autopsy cases were not included as cases in the analysis and were censored at the date of death. Fifteen men who were diagnosed with prostate cancer before the date of blood draw were excluded. For two cases, the date of diagnosis could not be determined, and these were eliminated from the analysis. A total of 62 men diagnosed with prostate cancer after the blood draw remained for analysis. The mean time from the blood draw between 1984 and 1990 to the diagnosis of prostate cancer was 5.6 ± 3.3 y. Of the cases for which clinical stage was documented ($n = 26$), 88.5% were stage B and 11.5% were stage C or D. Gleason scores were documented for 50 of 62 cases, and 70% of those cases had Gleason scores lower than 7.

Statistical analysis

Follow-up time was determined from the date of blood draw between 1984 and 1990 to the date of diagnosis of prostate cancer for each case. Because participants are seen only at the National Institute on Aging approximately every 2 y, follow-up time for non-cases was calculated from the date of blood draw to the date of their last visit if the visit was after January 1, 1999, 3 y after their last visit if the visit was before January 1, 1999, date of diagnosis of cancer, date of death, or the end of follow-up (November 26, 2001), whichever came first. Participants who were diagnosed with a cancer other than prostate cancer were censored at the time of cancer diagnosis because their plasma levels of vitamin C may have been altered subsequently due to changes in diet or due to therapy after diagnosis.

Using the distribution of plasma vitamin C concentrations in the entire cohort of men eligible for this analysis, quartiles of plasma vitamin C were generated. Cox proportional hazards regression models [16] were used to estimate the relative risk (RR) and 95% confidence interval (95% CI) for quartiles of plasma vitamin C. Age was included in the model as a continuous variable, and cigarette smoking status, body mass index, and circulating cholesterol concentration (as a surrogate for dietary fat intake) were evaluated separately as possible confounders. To test for trend, the median plasma vitamin C concentration for each quartile was entered as a continuous variable into the model. In a subanalysis, we excluded men who at the time of blood draw reported using multivitamins or vitamin C supple-

ments. All analyses were performed with SAS 8.1 (SAS Institute, Cary, NC, USA).

Results

Baseline characteristics of the cohort by quartiles of plasma vitamin C are displayed in Table 1. Men in the lowest quartile of plasma vitamin C were more likely to be current cigarette smokers and to have a higher mean body mass index compared with men in the higher quartiles. Although only a small percentage of men reported at the time of blood draw that they occasionally or regularly used multivitamins (3%) or vitamin C supplements (11%), men in the higher quartiles of plasma vitamin C were more likely to take multivitamins and vitamin C supplements than men in the lower quartiles. The median plasma vitamin C concentration for the cohort was 1.17 mg/dL, which is in the normal to high range for older men, and the median age at the time of blood draw was 63.6 y.

As presented in Table 2, prediagnostic plasma vitamin C concentrations were not inversely associated with subsequent prostate cancer. The results did not change with adjustment for cigarette smoking or with exclusion of current smokers from the analysis. Adjustment for body mass index strengthened the association between plasma vitamin C and prostate cancer, although the estimate was not statistically significant (comparing extreme quartiles, RR 1.42, 95% CI 0.67 to 3.01, P for trend = 0.21). Adjustment for plasma cholesterol did not alter the association (comparing extreme quartiles, RR 1.27, 95% CI 0.61 to 2.62, P for trend = 0.35). Exclusion of men who occasionally or regularly used multivitamins or vitamin C supplements attenuated the vitamin C and prostate cancer association (comparing extreme quartiles, RR 1.18, 95% CI 0.51 to 2.74, P for trend = 0.54).

Because a person's usual vitamin C intake may change over the long-term, in an alternate analysis we restricted the cases to those diagnosed within 5 y of blood draw. No association was observed (comparing extreme quartiles, RR 1.04, 95% CI 0.40 to 2.70, P for trend = 0.72). To decrease the likelihood that extant but not yet diagnosed prostate cancers or prodromal symptoms influenced plasma vitamin C concentration, we excluded cases diagnosed within 2 y of blood draw; the association remained in the positive direction (comparing extreme quartiles, RR 1.37, 95% CI 0.63 to 2.99, P for trend = 0.30).

There was little evidence of an association between plasma vitamin C concentrations and prostate cancer in men who were at least 70 y old at the time of blood draw (higher than versus no higher than the median, RR 0.89, 95% CI 0.40 to 1.98, P = 0.77). However, in men who were younger than 70 y at blood draw, the risk of prostate cancer was greater for men with higher plasma vitamin C levels (at least as high as versus lower than the median, RR 1.88, 95% CI 0.96 to 3.69, P = 0.07).

Discussion

We did not observe an inverse association between prediagnostic plasma vitamin C concentration and subsequent prostate cancer in this study, which was powered to detect clinically important moderate to strong associations. Our finding is consistent with that of Eichholzer et al. [4], who found no inverse association between plasma vitamin C and fatal prostate cancer in small cohort of working men in Switzerland, and with that of Huang et al. [5] who observed no significant trend between plasma ascorbic acid concentrations and prostate cancer risk in a community-based cohort in Maryland. Like our study, these studies used prediagnostic blood samples to assess the association between plasma vitamin C and prostate cancer, thus eliminating the possibility that the observed associations were due to changes in diet after cancer diagnosis. The majority of

Table 1
Baseline characteristics of the cohort by quartiles of plasma vitamin C, Baltimore Longitudinal Study of Aging

	Quartiles* of plasma vitamin C			
	1	2	3	4
Plasma vitamin C (mg/dL)				
Median	0.83	1.07	1.25	1.47
Range	0.15–0.98	0.99–1.16	1.17–1.35	1.36–2.58
Age (y), mean ± SD	60.2 ± 17.1	61.6 ± 15.0	62.6 ± 14.6	61.3 ± 17.0
BMI (kg/m^2), mean ± SD	26.8 ± 4.6	26.2 ± 3.1	25.5 ± 3.4	24.9 ± 3.4
Cigarette smoking status (%)				
Never	35.7	44.9	35.9	39.0
Former	48.4	50.0	55.0	50.4
Current	15.9	5.1	9.2	10.6
Vitamin supplement use (%)				
Multivitamin	1.6	1.7	5.3	3.3
Vitamin C	1.6	11.9	12.2	18.7

BMI, body mass index; SD, standard deviation

* Quartiles determined using plasma levels of vitamin C from the entire analytic cohort.

Table 2
Relative risks and 95% confidence intervals of prostate cancer by quartiles of plasma vitamin C, Baltimore Longitudinal Study of Aging

	Quartiles* of plasma vitamin C				Trend, *P*
	1	2	3	4	
Median (mg/dL)	0.83	1.07	1.25	1.47	
No. of cases	13	12	20	17	
No. of person-years	1149	1147	1163	1084	
Age-adjusted RR	1.0	0.87	1.37	1.31	0.29
95% CI	—	0.40–1.90	0.68–2.77	0.63–2.70	

CI, confidence interval; RR, relative risk
* Quartiles determined using plasma levels of vitamin C from the entire analytic cohort.

cohort and case-control studies examining dietary risk factors similarly have not observed a significant association between dietary intake of vitamin C and prostate cancer [6–9,17–21]. Although two retrospective case-control studies reported a significant inverse relation between dietary intake of vitamin C and prostate cancer [10,11], because of their design these studies could have been subject to recall bias.

One retrospective study associated high dietary intake of vitamin C with a statistically significant increased risk of prostate cancer in men 70 y and older but not in men younger than 70 y [22]. However, methodologic issues, such as recall bias and misclassification of vitamin C intake due to the brevity of dietary interview, could be explanations for these results. Our study did not observe a high risk for prostate cancer in older men with higher vitamin C plasma levels. There was some evidence to suggest that young men with higher vitamin C plasma concentrations were at an increased risk for prostate cancer, but we cannot preclude chance as an explanation for this observation.

Although the results of this study were not statistically significant, they were compatible with a possible small positive association between plasma vitamin C and prostate cancer risk. There is some evidence that high plasma levels of vitamin C may not afford protection against prostate carcinogenesis. Vitamin C is a redox chemical that is capable of providing protection against oxidative damage under certain conditions but is able to act as a pro-oxidant under other circumstances [23]. Although pro-oxidant effects of vitamin C have not been observed in vivo, in vitro high levels of vitamin C can increase the generation of free radicals in the presence of high stores of iron [24]. In addition, large doses of vitamin C have been shown to increase sister chromatid exchanges in Chinese hamster ovary cells [25]. Although the results were not statistically significant, three prospective studies also reported a small positive association between dietary intake of vitamin C and prostate cancer [7–9].

Several aspects of this study merit discussion. First, vitamin C is a water-soluble vitamin, and a single plasma measurement represents recent, rather than long-term, vitamin C intake. It is possible that long-term plasma concentrations may be more relevant for prostate cancer risk. With only one plasma level per person, we were unable to assess lifelong plasma vitamin C concentrations in this study. Second, high plasma vitamin C concentrations may provide a beneficial effect only for men with high levels of oxidative stress, such as cigarette smokers. We may have been unable to detect an inverse association in this population due to the low prevalence of current smokers in our study (10.2%). Third, as with any epidemiologic study, the results of our study may have been confounded by correlates of plasma vitamin C that are associated with prostate cancer. Adjustment for cigarette smoking, body mass index, or plasma cholesterol concentration as a surrogate for fat intake did not eliminate the suggestion of a positive association that we observed. Very few risk factors (e.g., age, race, and family history) have been convincingly demonstrated to be associated with total prostate cancer, making it less likely that confounding by factors unaccounted for in the analysis would explain our findings. Fourth, we attempted to minimize the influence of an existing but not yet diagnosed prostate cancer on plasma vitamin C concentration by exclusion of cases diagnosed close in time to blood draw, which did not attenuate the vitamin C and prostate cancer association, and by exclusion of men who used vitamin C–containing supplements, which did attenuate the association, although it was still in the positive direction.

Although this study was conducted in a subset of the entire BLSA cohort, the men should not have been selected to alter or obscure the true association between plasma vitamin C and prostate cancer risk. All the men included in this study had plasma vitamin C levels measured as part of an unrelated nutritional assessment. The prospective nature and relatively long interval between assessment and outcome make it unlikely that selection factors could have affected the observed results of our study.

Because most participants in our study were well nourished and had normal plasma concentrations, we may have been unable to detect an increased risk of prostate cancer associated with very low levels of plasma vitamin C. However, it is unlikely that an inverse association was missed because of lack of high vitamin C exposure. In this study, the median plasma vitamin C levels in the top (1.46 mg/dL) and bottom (0.80 mg/dL) quartiles were compatible with the normal to high-normal range (0.6 to 1.4 mg/dL) [26].

Higher plasma vitamin C concentrations are difficult to achieve because of the enhanced renal excretion of ascorbic acid when plasma concentrations exceed 1.2 mg/dL [23]. If plasma vitamin C concentration only has a modest effect on prostate cancer risk, then our sample may have been too small to detect the association. This study was powered to detect only clinically relevant, moderate to strong associations.

In conclusion, our prospective study suggests that higher plasma vitamin C concentrations are not associated with a lower risk of subsequent prostate cancer in well-nourished adult men.

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